

WHAT IS CLAIMED IS:

1. A method for producing an antibody dimer comprising:
 - (i) obtaining or constructing a DNA molecule that encodes an antibody molecule heavy chain that has a desired binding specificity and
5 introducing at least one cysteine codon therein via recombinant DNA mutagenesis;
 - (ii) expressing said DNA molecule in a suitable host cell, or expression system, together with a DNA molecule that encodes an antibody molecule light chain of desired specificity, to produce an antibody molecule
10 containing said introduced cysteine residue;
 - (iii) purifying said antibody molecule from said host cell or expression system;
 - (iv) contacting said purified antibody molecule with an amount of a suitable reducing agent sufficient to partially reduce the intra or inter
15 molecular disulfide bonds of said antibody molecule and thereby enhance the function of antibody dimers;
 - (v) allowing sufficient time for the dimerization reaction to

(vi) optionally terminating the reducing reaction by the addition of cysteine or after thiol blocking reagent.

2. An IgG/IgG dimer produced by the method of Claim 1.

3. The IgG/IgG dimer of Claim 2, wherein said IgG/IgG dimer is a
5 homodimer.

4. The IgG/IgG dimer of Claim 2 which is a heterodimer.

5. The method of Claim 1, which results in an IgG/IgG dimer capable
of activating components of the complement system.

6. The method of Claim 1, which results in an IgG IgG dimer that
10 comprises the ability to activate and kill cells via the complement cascade.

7. The method of Claim 1, which results in an IgG IgG dimer that is

8. The method of Claim 7, which results in an IgG/IgG dimer that binds to Fcγ receptors on host immune cells.

9. The method of Claim 2 which results in an IgG/IgG dimer capable of initiating programmed cell death (apoptosis).

5 10. The dimer of Claim 3, wherein said homodimer is a an anti-CD20 homodimer.

11. The dimer of Claim 10, wherein said anti-CD20 dimer is a C2B8 homodimer.

12. The dimer of Claim 3, wherein said homodimer is an anti-CD23
10 dimer.

13. The dimer of Claim 12, wherein said anti-CD23 dimer is a p3E8 homodimer.

14. The dimer of Claim 2, wherein said dimer is reactive against the CD23 antigen and/or the CD20 antigen.

15. A method for treating cancer comprising contacting cancer cells with an antibody dimer produced according to Claim 1.

5 16. A method for treating cancer comprising contacting cancer cells with an Ab dimer according to Claim 10.

17. A method for treating an allergic disorder comprising administering an effective amount of the p5E8 homodimer of Claim 13 to a patient in need of such treatment.

10 18. The method of Claim 17, wherein said disorder is selected from the group consisting of allergic asthma, allergic bronchopulmonary *aspergillosis*, allergic rhinitis atopic dermatitis, Chrones disease, Graves disease, food allergy, and allergic contact dermatitis..

19. The method of Claim 16, wherein said cancer is CLL or B-cell lymphoma.

20. A pharmaceutical composition comprising an antibody dimer produced according to Claim 1, and a pharmaceutically acceptable carrier.

5 21. A method of treatment comprising administering the pharmaceutical composition of Claim 8 to a patient in need of such treatment.

22. A method for treating an autoimmune disorder comprising administering an effective amount of an IgG/IgG dimer produced according to Claim 1 to a patient in need thereof.

10 23. The method of Claim 22, wherein said IgG/IgG dimer is an anti-gp39 dimer.

24. A method for producing an antibody dimer comprising:

25. a) selecting a heavy chain and a light chain having binding specificity and

introducing at least one cysteine codon therein via recombinant DNA technologies;

(ii) expressing said DNA molecule in a suitable host cell, or expression system, together with a DNA molecule that encodes an antibody molecule light chain of desired binding specificity, to produce an antibody
5 molecule containing said introduced cysteine residue;

(iii) purifying said antibody molecule from said host cell or expression system;

(iv) contacting said purified antibody molecule with an amount
10 of a suitable reducing agent sufficient to partially reduce the intra or inter molecular disulfide bonds of said antibody molecule and thereby enhance the function of antibody dimers;

(v) adding a thiol reactive group introduced on another antibody molecule which does not have a cysteine group introduced therein and allowing
15 sufficient time for the dimerization reaction to proceed; and

(vi) optionally terminating the reducing reaction by the addition of cysteine.

25. The method of Claim 24, wherein the thiol reactive group is a maleimido group.

26. The method of Claim 24, wherein the thiol reactive group is a dithiopyridal group.

5 27. The method of Claim 24, wherein the thiol reactive group is a reactive thiol.

28. An IgG/IgG dimer produced by the method of Claim 23, wherein said IgG's are of the same or different IgG subclass.

29. The method of Claim 24, wherein said dimer comprises MAb
10 molecules of different isotypes.

30. The method of Claim 24, wherein said IgG/IgG dimer is a homodimer.

31. The method of Claim 30, wherein said homodimer is a C2B8 homodimer.

32. The method of Claim 30, wherein said homodimer is a p5E8 homodimer.

5 33. The method of Claim 30, wherein said homodimer is reactive against CD23 antigen.

34. The method of Claim 24, wherein said IgG/IgG dimer is a heterodimer having binding specificity for two different epitopes.

35. The method of Claim 34, wherein said heterodimer is reactive
10 against the CD20 and CD23 antigen.

36. The method of Claim 35, wherein said heterodimer is a C2B8/p5E8 heterodimer.

Method for producing an antibody, cancer comprising

- (i) obtaining a DNA molecule that encodes an antibody molecule heavy chain that has a desired binding specificity and introducing at least one cysteine codon therein via site specific mutagenesis;
- (ii) expressing said DNA molecule in a suitable host cell,
5 together with a DNA molecule that encodes an antibody light chain, to produce an antibody molecule containing said introduced cysteine residue;
- (iii) purifying said antibody molecule from said host cell;
- (iv) contacting said purified antibody molecule with an amount of a suitable reducing agent sufficient to partially reduce the intra or inter
10 molecule disulfide bonds of said antibody molecule and thereby enhance the function of antibody dimers;
- (v) cross-linking the reduced antibody molecules using a BIS-maleimido cross-linker;
- (vi) optionally terminating the reducing reaction by the addition
15 of cysteine.

38. A method for treating cancer comprising contacting cancer cells

39. A method for treating an allergic disorder comprising administering an effective amount of a dimer according to Claim 32 to a patient in need of such treatment.

40. The method of Claim 38, wherein said cancer is CLL or B-cell
5 lymphoma.

41. A pharmaceutical composition comprising an IgG/IgG dimer
according to Claim 28, and a pharmaceutically acceptable carrier.

42. A method for treating cancer comprising administering the pharmaceutical composition of Claim 41 to a patient in need of such treatment.

10 43. A method for treating an autoimmune disorder comprising administering an IgG/IgG dimer according to Claim 10, to a patient in need of such treatment.

44. A method for treating an allergic disorder comprising administering an effective amount of an IgG/IgG dimer according to Claim 15 to a patient in need of such treatment.

45. A method for producing an IgG/IgG dimer comprising genetically engineering a MAb to introduce a cysteine molecule placed which inhibits or prevents formation of an intramolecular disulfide bridge between sister heavy chains on the same antibody molecule.

46. An IgG/IgG dimer produced by the method of Claim 44.

WHAT IS CLAIMED IS:

1. A method for producing an antibody dimer comprising:
- (i) obtaining or constructing a DNA molecule that encodes an antibody molecule heavy chain that has a desired binding specificity and
- 5 introducing at least one cysteine codon therein via recombinant DNA mutagenesis;
- (ii) expressing said DNA molecule in a suitable host cell, or expression system, together with a DNA molecule that encodes an antibody molecule light chain of desired specificity, to produce an antibody molecule
- 10 containing said introduced cysteine residue;
- (iii) purifying said antibody molecule from said host cell or expression system;
- (iv) contacting said purified antibody molecule with an amount of a suitable reducing agent sufficient to partially reduce the intra or inter
- 15 molecular disulfide bonds of said antibody molecule and thereby enhance the function of antibody dimers;

proceed; and

(vi) optionally terminating the reducing reaction by the addition of cysteine or after thiol blocking reagent.

2. An IgG/IgG dimer produced by the method of Claim 1.

3. The ~~IgG/IgG~~ dimer of Claim 2, wherein said ~~IgG/IgG~~ dimer is a
5 homodimer.

4. The IgG/IgG dimer of Claim 2 which is a heterodimer.

5. The method of Claim 1, which results in an IgG/IgG dimer capable of activating components of the complement system.

Pat. 6,111,125, 6,000,081, 3,913
D12,776, 124,740, 651,504
D12,776, 124,486, 435,567
D12,776, 377,715, 543,546
gamma globulin

6. The method of Claim 1, which results in an IgG/IgG dimer that
10 comprises the ability to activate and kill cells via the complement cascade.

capable of binding to Fcγ receptors on cytotoxic effector cells.

D12,776, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000

D12,776, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000

8. The method of Claim 7, which results in an IgG/IgG dimer that binds to Fcγ receptors on host immune cells.

9. The method of Claim 2 which results in an IgG/IgG dimer capable of initiating programmed cell death (apoptosis). ~~caspase~~ caspase?

64.335,139,160

5 10. The dimer of Claim 3, wherein said homodimer is a an anti-CD20 homodimer.

11. The dimer of Claim 10, wherein said anti-CD20 dimer is a C2B8 homodimer.

10 12. The dimer of Claim 3, wherein said homodimer is an anti-CD23 dimer.

13 The dimer of Claim 12, wherein said anti-CD23 dimer is a C2B8

14. The dimer of Claim 2, wherein said dimer is reactive against the CD23 antigen and/or the CD20 antigen.

15. A method for treating cancer comprising contacting cancer cells with an antibody dimer produced according to Claim 1.

5 16. A method for treating cancer comprising contacting cancer cells with an Ab dimer according to Claim 10.

17. A method for treating an allergic disorder comprising administering an effective amount of the p5E8 homodimer of Claim 13 to a patient in need of such treatment.

10 18. The method of Claim 17, wherein said disorder is selected from the group consisting of allergic asthma, allergic bronchopulmonary *aspergillosis*, allergic rhinitis atopic dermatitis, Crohn's disease, Graves' disease, Hashimoto's thyroiditis, multiple sclerosis, and rheumatoid arthritis.

19. The method of Claim 16, wherein said cancer is CLL or B-cell lymphoma.

20. A pharmaceutical composition comprising an antibody dimer produced according to Claim 1, and a pharmaceutically acceptable carrier.

5 21. A method of treatment comprising administering the pharmaceutical composition of Claim 8 to a patient in need of such treatment.

22. A method for treating an autoimmune disorder comprising administering an effective amount of an IgG/IgG dimer produced according to Claim 1 to a patient in need thereof.

10 23. The method of Claim 22, wherein said Ig/IgG dimer is an anti-gp39 dimer.

(1) obtaining or constructing a DNA molecule that encodes an antibody molecule; (2) inserting that DNA molecule into a host cell; and (3) culturing the host cell to produce the antibody molecule.

introducing at least one cysteine codon therein via recombinant DNA technologies;

(ii) expressing said DNA molecule in a suitable host cell, or expression system, together with a DNA molecule that encodes an antibody molecule light chain of desired binding specificity, to produce an antibody molecule containing said introduced cysteine residue;

(iii) purifying said antibody molecule from said host cell or expression system;

(iv) contacting said purified antibody molecule with an amount of a suitable reducing agent sufficient to partially reduce the intra or inter molecular disulfide bonds of said antibody molecule and thereby enhance the function of antibody dimers;

(v) adding a thiol reactive group introduced on another antibody molecule which does not have a cysteine group introduced therein and allowing sufficient time for the dimerization reaction to proceed; and

(vi) optionally terminating the reaction by adding a thiol blocking agent.

25. The method of Claim 24, wherein the thiol reactive group is a maleimido group.

26. The method of Claim 24, wherein the thiol reactive group is a dithiopyridal group.

5 27. The method of Claim 24, wherein the thiol reactive group is a reactive thiol.

28. An IgG/IgG dimer produced by the method of Claim 23, wherein said IgG's are of the same or different IgG subclass.

29. The method of Claim 24, wherein said dimer comprises MAb
10 molecules of different isotypes.

30. The method of Claim 24, wherein said dimer comprises

31. The method of Claim 30, wherein said homodimer is a C2B8 homodimer.

32. The method of Claim 30, wherein said homodimer is a p5E8 homodimer.

5 33. The method of Claim 30, wherein said homodimer is reactive against CD23 antigen.

34. The method of Claim 24, wherein said IgG/IgG dimer is a heterodimer having binding specificity for two different epitopes.

35. The method of Claim 34, wherein said heterodimer is reactive
10 against the CD20 and CD23 antigen.

36. The method of Claim 35, wherein said heterodimer is a C2B8/p5E8

37. A method for producing an antibody dimer comprising:

(i) obtaining a DNA molecule that encodes an antibody molecule heavy chain that has a desired binding specificity and introducing at least one cysteine codon therein via site specific mutagenesis;

(ii) expressing said DNA molecule in a suitable host cell,
5 together with a DNA molecule that encodes an antibody light chain, to produce an antibody molecule containing said introduced cysteine residue;

(iii) purifying said antibody molecule from said host cell;

(iv) contacting said purified antibody molecule with an amount of a suitable reducing agent sufficient to partially reduce the intra or inter
10 molecule disulfide bonds of said antibody molecule and thereby enhance the function of antibody dimers;

(v) cross-linking the reduced antibody molecules using a BIS-maleimido cross-linker;

(vi) optionally terminating the reducing reaction by the addition
15 of cysteine.

with the IgG IgG dimers of Claim 28.

39. A method for treating an allergic disorder comprising administering an effective amount of a dimer according to Claim 32 to a patient in need of such treatment.

40. The method of Claim 38, wherein said cancer is CLL or B-cell lymphoma.

41. A pharmaceutical composition comprising an IgG/IgG dimer according to Claim 28, and a pharmaceutically acceptable carrier.

42. A method for treating cancer comprising administering the pharmaceutical composition of Claim 41 to a patient in need of such treatment.

43. A method for treating an autoimmune disorder comprising administering an IgG-IgG dimer according to Claim 10, to a patient in need of such treatment.

44. A method for treating an allergic disorder comprising administering an effective amount of an IgG/IgG dimer according to Claim 15 to a patient in need of such treatment.

45. A method for producing an IgG/IgG dimer comprising genetically engineering a MAb to introduce a cysteine molecule placed which inhibits or prevents formation of an intramolecular disulfide bridge between sister heavy chains on the same antibody molecule.

46. An IgG/IgG dimer produced by the method of Claim 44.